Aim: The aim of this study was to establish an immortalized normal human articular chondrocyte line which could be useful for a better understanding of cell molecular mechanisms relevant for the development of new therapeutic approaches in rheumatic diseases.

Methods: Chondrocytes from human adult articular healthy cartilage were transfected in primary culture with a plasmid containing two human papilloma virus type 16 (HPV-16) early function genes: E6 and E7, using the highly efficient cationic liposome-mediated (lipofection) procedure. The established chondrocyte cell line was examined in monolayer and in two culture conditions that were described to re-induce differentiated characteristics: culturing in a serum-free defined medium supplemented with an insulin-containing serum substitute and seeding on a hyaluronan-based non-woven structured biomaterial.

Results: Normal human articular chondrocytes were efficiently transfected leading to the establishment of an immortalized cell line as confirmed by HPV-16 E7 mRNA detection. These cells lost the cartilage characteristic phenotype during their growth in monolayer conditions, as observed for chondrocytes in primary culture, showing the suppression of type II collagen mRNA expression and stable synthesis of collagen type I. However, they were able to re-express type II collagen under the two defined cultured conditions we used, still maintaining type I collagen expression.

Conclusions: The cell line that we obtained may be a useful tool for increasing our knowledge of the genetic and biochemical events involved in time processes of cartilage growth and differentiation. Moreover, it appears to be a suitable model for pharmacological and toxicological studies related to rheumatic diseases relevant to humans.

PB47

EXPRESSION OF TRANSDUCED HSP70 GENE PROTECTS CHONDROCYTES FROM APOPTOSIS

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Aim: The aim of the study was to investigate the efficacy of the heat shock protein 70 (HSP70) gene transfer to chondrocytes apoptosis.

Methods: Two adenovirus vectors that contain wild-type (AxSHEwt) or mutant-type (AxSHEmt) HSP 70 gene with SRa promoter were constructed. Chondrocytes were obtained from articular cartilage of Japanese white rabbits (2kg), and cultured for 1 week. After transfection by adenovirus vectors, Apoptosis was induced by 0.5mM sodium nitroprusside dihydrate (SNP). The effect of adenovirus vector mediated HSP 70 gene transfer on the apoptosis was investigated using lactate dehydrogenase (LDH) activity assay and the Hoechst 33342 staining.

Results: In the LDH activity assay, the absorbance levels were 480.0±89.1 in the controls, 478.8±15.4 in AxSHEmt transduced cells and 462.8±22.3 in AxSHEwt transduced cells. The absorbance levels in the AxSHEwt were lower than other two groups. The Hoechst 33342 staining assay, revealed that the percentages of apoptotic cells were 33.9±2.8% (control), 36.1±2.8% (AxSHEmt), and 25.0±3.9% (AxSHEwt). The percentage of apoptotic cell in AxSHEwt transduced cell was significantly lower in than in other two groups ($p<O.O5$).

Conclusions: HSP 70 has been known to protect cells from various stresses. Recent studies demonstrated that the apoptosis of articular chondrocytes plays an important role in the pathogenesis of osteoarthritis (OA) and that nitric oxide (NO) could stimulate this process. This study showed that the HSP70 gene transfer protected chondrocytes from the apoptosis induced by NO. This suggests that the HSP 70 gene transfer to chondrocytes could be useful for the treatment for OA.

PB48

Abstract PB48 has not been assigned

PB49

CORRELATION OF SERUM LEVELS OF COMP AND YKL-40 WITH SCINTIGRAPHIC SCAN IN KNEE OA

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Aim: To correlate levels of two serological markers of cartilage degradation and synovial inflammation with bone scintigraphy.

Methods: The study included 34 patients with knee OA. Scintigraphy was performed with 600 MBq ^{99m} TC bisphosphonate. COMP was measured by inhibition ELISA with monoclonal antibodies 17-C 10 (1), YKL-40 was measured by commercially available kit (Metra Biosystem). Semiquantitative assessment of scintigraphy was performed using: "scintigraphic index" (four compartments of each knee joint, intensity 0-3, total index 0-12). Statistics - Spearman correlation coefficient.

Results: No correlation was found between scintigraphic indices of either or late phase and serum levels of COMP or YKL-40. COMP levels of patients with negative scan were lower (2.99 ± 0 .71 λg/ml) than those of patients with unilateral (4.07 ± 2.18 λg/ml) or bilateral (3.81 ± 1.41 λg/ml) positive scan but the differences were not significant. In late phase of scan all tested knees were positive. Serum COMP levels in patients with unilateral positive scan tended to be lower (3.54 vs 3.9 λg/ml, $p = 0.07$) than in bilateral positive scan.

Conclusion: We found no correlation between scintigraphic scan with ^{99m} TC bisphosphonate and serum levels of COMP and YKL 40 in knee OA.

Reference

1. Vilim et al. *Arch.Biochem Biophys* 1997; 341: 8-16

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PB50

HYALURONIC ACID (HA) IN OSTEOARTHRITIS (OA) SYNOVIAL FLUID (SF): RELATIONSHIP WITH OTHER INFLAMMATORY AND OSTEOARTHRITIC MARKERS

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Aim: To determine the HA levels in SF of OA knees and to evaluate possible relationship with other SF OA markers.

Material and Methods: Fifty SF obtained from knee OA (ACR criteria and Kellgren-Lawrence grade II or III) were assayed for HA, phospholipase A2 (PLA2), cartilage oligomeric matrix protein (COMP), prostaglandins E2 (PGE2), , YKL4O, active collagenase and total proteins determination. Correlation between markers were studied using non-parametric Spearman test.

Results: Mean SF HA (±SD) was 2.24±0.60 mg/ml. There was no significant difference between KL grades II (2.31±0.60) and III (2.35±0.67); p=0.91. HA SF level was correlated with PGE2 (r=0.58, p=0.001), PLA2 (r=0.32, p=0.02), COMP (r=0.29, p=0.04) and active collagenase (r=0.31 1, p=0.02). HA was unrelated to YKL4O (r=0.25, p=0.07) and SF total proteins (r=0.01, p=O.93). Other positive correlations between PLA2 and YKL4O (r=0.34, p=0.0l), active collagenase and COMP (r=0.28, p=0.04) and PGE2 (r=0.34, p=0.0l) were found.

Conclusion: These results show that SF HA is an inflammatory marker of OA joint which is highly correlated to other markers of both inflammatory process (PGE2 and PLA2) and cartilage metabolism (collagenase and COMP).

PB51

CORRELATION OF CD44, HSP7O, AND HYALURONAN IN SYNOVIAL TISSUE WITH CLINICAL AND RADIOLOGICAL SCORES

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Aim: Correlation of clinical and radiological scores with synovial expression of CD44, HSP70, and hyaluronan in osteoarthritis.

Material and Methods: Synovial tissue from the knee joint of 38 patients with different grades of primary osteoarthritis (according to Kellgren) were collected, fixed in 10% buffered formalin (pH 7.0), and embedded in paraffin. After decalcification specimens were cut in 3.5λm sections and analyzed for CD44H, CD44v5, HSP7O, and hyaluronan using monoclonal antibodies. Antigen detection was performed using the PAP method. Hematoxylin/eosin, alcian blue, and safranin 0 was used for histological staining. Analysis of specimens was carried out five times by two independent examiners. Histopathomorphological results were classified according to Pelletier. Immunhistochemical staining was graded into 0 = no, 1 = low, 2 = moderate, 3 = intensive. For clinical examination Visual Analog Scale (VAS), Knee Society Score (KSS), and Lequesne Score were used.

Results: Clinical examinations revealed 8.7 ± 1.2 (mean ± SD) points for VAS, 70.68 ± 41.45 (mean ± SD) points for KSS, and 16.88 ± 6.74 (mean ± SD) points for the Lequesne Score. Analysis according to Pelletier achieved 6.9 ± 1.32 (mean ± SD) points. Immunhistochemical staining was 1.53 ± 0.89 (mean ± SD) for CD44H 113 ± 0.99 (mean ±SD) for CD44v5, 0.39 ± 0.59 (mean ± SD) for hyaluronan, and 1.08 ±1.15 (mean ± SD) for HSP70. No significant differences were found between the grades of osteoarthritis. Correlation analysis was significant (p *<0.05)* for age and Pelletier classification (0.323), KSS and Lequesne Score (0.329), HSP70 and age (0.375), CD44H and BMI (0.386), CD44v5 and age (0.409), hyaluronan and VAS (0.345), CD44H and hyaluronan (0.362), and Lequesne Score and hyaluronan (0.329). A significance level of p <0.01 was only achieved for VAS and KSS.

Conclusions: The results indicate that expression of HSP70 and CD44v5 is rather associated with age than with clinical parameters. In contrast, the correlation of hyaluronan with CD44H and clinical symptoms points to a role for hyaluronan and probably its receptor CD44H in osteoarthritis.

PB52

SERUM C-TELOPEPTIDE OF TYPE I COLLAGEN IN EROSIVE OSTEOARTHRITIS OF THE HANDS

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Aim: The aim of the study was to assess the serum levels of CTx in patients with erosive osteoarthritis of the hands, a disease with an elevated bone turnover.

Patients and Methods: Samples for serum C-telopeptide of type I collagen (CTx), were obtained from 19 patients (18 women and 1 man; age 49,79 years) with erosive osteoarthritis of the hands, and from 23 patients (22 women and 1 man; age 48-90 years) with osteoporosis. The mean duration of diseases was about 7 years. Admission criteria for EOA was the radiological evidence of central erosions in 2-6 interphalangeal joints. Osteoporotic patients had a z-score of -2.5 SD at the examination with DEXA Lunar-Expert densitometer. The serum Cross Laps One Step ELISA kit was employed.

Results: The serum CTx concentration was 3376+/-1622 pmol/litre (mean +/SD) in osteoporotic patients and 4720+/-1895 pmol/litre in osteoarthritic patients. A significant difference was observed between the two groups (P<0.01). Osteoporotic patients 7/23 (30.4%) presented elevated CTx values versus osteoarthritic patients where abnormal CTx values were 12/19 (63.1%); the difference was statistically significant (P<0.0 1).

Fig. 1. Distribution of mean values of serum CTx of the osteoporotic and osteoarthritic patients

Conclusions: Serum C-telopeptide of type I collagen (CTx) was found elevated in different conditions with high bone turnover expecially in osteoporotic patients. The study demonstrates than the patients affected by erosive osteoarthritis of the hands presented serum levels of CTx more elevated than osteoporotic patients

PB53

BIOLOGICAL CHARACTERISTICS OF THE RAPIDLY DESTRUCTIVE OSTEOARTHRITIS OF THE HIP: A CASE-CONTROL STUDY

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Objective : To determine the biological characteristics of the rapidly destructive hip osteoarthritis (RDHOA) in relation to common hip OA.

Methods: Case-control study. Three groups: group I : 27 patients suffering from RDHOA (annual joint space narrowing JSN>2 mm per year); group II: 27 patients with slow progressive hip OA (annual JSN<O.50 mm/year) matched by age and sex; group III : 27 patients with slow progressive hip OA matched by the joint space thickness. The studied serum markers were: C Reactive Protein (CRP) using high sensitive nephelemetry, Cartilage Oligomeric Matrix Protein (COMP), hyaluronic acid (HA), tissue inhibitor of metalloproteinase type 1 (TIMP-1), Matrix metalloproteinase type 1 (MMP-1), type I collagen C-terminal propeptide